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Recycling of textile bleaching effluents for dyeing using immobilized catalase

Silgia A. Costa[1], Tzanko Tzanov[1], Filipa Carneiro[1], Georg M. Gübitz[2] & Artur Cavaco-Paulo[1,*]
[1]*Department of Textile Engineering, University of Minho, 4810 Guimarães, Portugal*
[2]*Department of Environmental Biotechnology, Graz University of Technology, 8010 Graz, Austria*
[*]*Author for correspondence (Fax: +351 253 510293; E-mail: artur@det.uminho.pt)*

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Abstract

Catalase was immobilized on alumina carrier and crosslinked with glutaraldehyde. Storing stability, temperature and pH profiles of enzyme activity were studied in a column reactor with recirculation and in a batch stirred-tank reactor. The immobilized enzyme retained 44% of its activity at pH 11, 30 °C and 90% at 80 °C, pH 7. The half-life time of the immobilized catalase was increased to 2 h at pH 12, and 60 °C. Acceptable results were achieved when the residual water from the washing process of H_2O_2-bleached fabrics was treated with the immobilized enzyme and then reused for dyeing.

Introduction

The problems of process costs and pollution of residual waters in the textile industry require increasing attention due to the new ecological regulations and from an economic point of view (Dickinson 1984). H_2O_2 is a bleaching agent that is widely used in industrial textile processes (Spiro & Griffith 1997). Its removal in the washing of cotton fabrics after bleaching consumes large amounts of water and energy (Weck 1991, Stöhr & Petry 1995) in order to avoid subsequent problems with O_2-sensitive dyes (Jensen 2000). Catalases can be used to degrade H_2O_2 (Vasudevan & Thakur 1994, Emerson *et al.* 1996) so as to decrease water consumption or to recycle the washing liquor for dyeing. Major problems in using enzymes arise from the high temperature and alkalinity of the bleaching and washing liquors. Previously, we have attempted to solve this problem by screening new thermoalkaliphilic microorganisms (Paar *et al.* 2001) or by using different enzyme stabilizers (Costa *et al.* 2001). Possible interactions between dye and protein (Tzanov *et al.* 2001a, b) indicate that the use of soluble catalase is inappropriate. The use of immobilized catalase would be an alternative (Costa *et al.* 2001, Amar *et al.* 2000). In this study, we have covalently immobilized a commercial catalase on alumina support using glutaraldehyde as a crosslinking agent. The objective of this study was to investigate the kinetics of the immobilized catalase, its operational stability and the process conditions, which would enable us to apply this system to treat washing liquors that might be reused for dyeing.

Materials and methods

Enzyme

Terminox Ultra 50L is a commercial catalase (EC 1.11.1.6) provided by AQUITEX – Maia, Portugal.

Catalase immobilization

Pellets and pastilles of Al_2O_3 (Aldrich), diam. 3 and 7 mm, respectively, were silanized in 4% (v/v) γ-aminopropyltriethoxy silane (Sigma) in acetone at 45 °C for 24 h. The silanized carriers were washed-off with distilled water and treated with 2% (v/v) aqueous glutaraldehyde (Aldrich) for 2 h at room temperature,

washed again and dried at 60 °C for 1 h. Five grams of support were then immersed in 25 ml crude enzyme preparation for 24 h at room temperature (Costa *et al.* 2001). The amount of immobilized protein was 18 mg g^{-1} pellets and 14 mg g^{-1} pastilles.

Activity assay for free and immobilized enzyme

The free catalase activity was measured spectrophotometrically at 240 nm following the degradation of 26 mM H_2O_2 in 50 mM potassium phosphate buffer, pH 7, at 30 °C. The activity was calculated using a molar absorption coefficient for H_2O_2 of 39.4 M cm (Aebi 1974). The immobilized enzyme activity was determined in thermostated column reactor, with recirculation (1 ml min^{-1}), and in a batch stirred-tank reactor with H_2O_2 from 0.015 M to 2 M, at pH 7 and 30 °C. The reactors were loaded with 5 g immobilized catalase (Costa *et al.* 2001) and the enzymatic reaction started with the feeding of the substrate solution through the column. Remaining H_2O_2 was determined by $KMnO_4$ titration and analytical test trips from Merck. Catalase activity was expressed in μmol min^{-1} mg^{-1} bound protein.

Protein assay

The amount of protein in the supernatant solution after immobilization was determined using the Bradford method. Bound protein was determined as a difference between initial and residual protein concentrations.

Washing liquor after bleaching

The residual water was taken from a textile finishing company (TAS, Portugal) after washing peroxide bleached (52.5 g H_2O_2 l^{-1} (50% v/v) cotton fabrics. The fabrics were washed in a washing machine with six consecutive boxes.

Dyeing conditions

The dyeing was performed using the reactive dye Evercion Blue HER (from Everlight Chem. Ind., Taiwan) at 1.5%, with Na_2SO_4 (50 g l^{-1}), and Na_2CO_3 (20 g l^{-1}). The dyeing baths were prepared using washing water, treated with immobilized enzyme. Dyeing experiments were carried out at 80 °C, for 60 min.

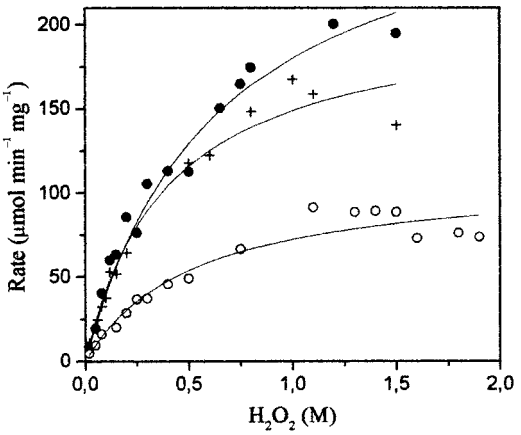

Fig. 1. Rate of H_2O_2 decomposition with immobilized catalase (μmol H_2O_2 min^{-1} mg^{-1} protein): pellets support in (●) column reactor and (+) batch stirred tank reactor, and pastille support in (○) column reactor. (Experiments performed in triplicate with less than 4% of error.)

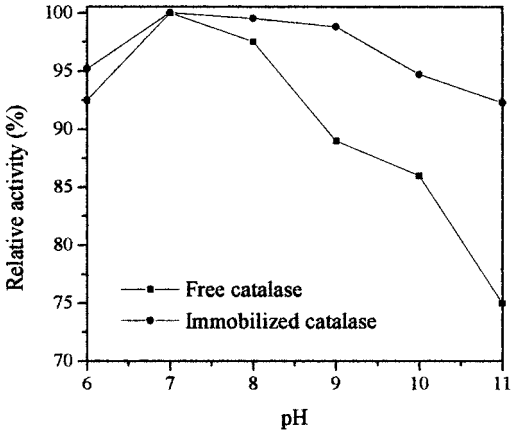

Fig. 2. Effect of pH on the activity of free and immobilized catalase. Activity measured with 26 mM H_2O_2, 30 °C, 2 h of incubation at pHs ranging from 6 to 12 (6–7 potassium phosphate; 8–9 Tris HCl; 10–12, sodium carbonate buffers at 50 mM). 100% correspond to activities of 12 μmol min^{-1} mg^{-1} protein for immobilized catalase and 8 μmol min^{-1} for free catalase. (Experiments performed in triplicate with less than 4% of error.)

Colour difference (ΔE^*)

The colour difference between fabric dyed in fresh water and dyed in catalase treated washing liquor was determined using a reflectance-measuring Datacolor apparatus Spectraflash 600, according to the CIELab colour difference concept at standard illuminant D_{65} (LAV/Spec. Excl., d/8, D_{65}/10°).

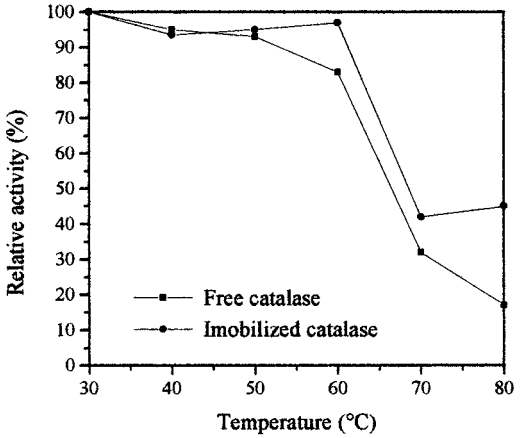

Fig. 3. Effect of the temperature on the activity of free and immobilized catalase. Activity measured with 26 mM H_2O_2in column reactor after incubation in 50 mM phosphate buffer pH 7, for 2 h. 100% correspond to activities of: 12 μmol min^{-1} mg^{-1} protein for immobilized catalase, and 8 μmol min^{-1} mg^{-1} for free catalase. (Experiments performed in triplicate with less than 4% of error.)

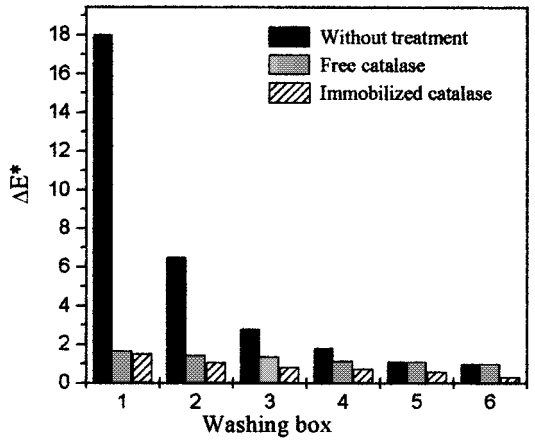

Fig. 4. Dyeing in after-bleaching washing liquors, treated with free and immobilized catalase. ΔE^*-colour difference between fabric dyed in fresh water and dyed in catalase treated washing liquor. (Experiments performed in triplicate with less than 4% of error.)

Results and discussion

Rate of substrate transformation with immobilized catalase

The rate of enzymatic reaction in the reactors increased with increasing concentrations of H_2O_2 (Figure 1). Above 1 M H_2O_2, the rate maintained constant. The catalase was inactivated possibly by excess of H_2O_2 or by formation of some organic peroxide. This substrate inactivation is normally associated with the modifications of heme prosthetic group of the enzyme (Lafuente *et al.* 1999).

pH and temperature dependence of catalase activity

Both free and immobilized catalase showed activity maximum at pH 7 (Figure 2). However a significant distinction can be made based on their pH sensitivity: the immobilized enzyme had a higher activity at alkaline pHs. The covalent binding of catalase on the support might improve the resistance of the enzyme against inactivation presumably by restricting the protein unfolding process as a result of the introduction of both intra- and inter-molecular crosslinks.

The improvement in the thermal stability of immobilized catalase, compared to the free enzyme, was clearly pronounced above 50 °C (Figure 3). The connection between support and biocatalyst can be obtained either by direct linkage between the components or via a link of differing length, a so-called spacer. The spacer molecule gives a greater degree of mobility to the coupled biocatalyst so that its activity can, under certain circumstances, be higher than if it is bound directly to the support. The glutaraldehyde used as crosslinking agent or spacer renders the protein structure more flexible and resistant to thermal denaturation (Duhalt 1999).

Storage stability of free and immobilized catalase

The thermal and pH stability of the catalase are basic criteria for its long-term industrial applications. The stability of both free and immobilized enzymes decreased with the increase in temperature and alkalinity (Table 1) but the immobilized enzyme was better at the highest pH and temperature studied.

Dyeing in after-bleaching washing liquor treated with free and immobilized catalase

The amount of H_2O_2, temperature and pH of the washing liquor from the different boxes of the washing machine decreased from the first to the last (No. 6) as follows: 0.059, 0.019, 0.009, 0.007, 0.005, 0.004 g l^{-1} H_2O_2; from 90 °C to 40 °C; pH from 11 to 4.5. Consequently, the colour yield in the subsequent dyeings using enzymatically – recycled water increased in the same order from the first to the last box (Figure 4). The dyeing in immobilized enzyme-treated residual water resulted in smaller colour difference compared to the dyeing in free enzyme-treated bath.

The significant improvement of dyeing in case of immobilized catalase could be explained by the

Table 1. The half-life times of free and immobilized catalase studied at 30 °C and 60 °C, pH 7 – 12. (Experiments performed in triplicate with less than 4% of error.)

Catalase	Temperature (°C)	Half-life time (h) at pH values					
		7	8	9	10	11	12
Free	30	612	387	362	192	102	8
	60	18	14	11	6	0.07	0.05
Immobilized on pellets	30	882	235	214	199	65	35
	60	287	109	71	61	16	2

greater efficiency of the immobilized catalysts at the corresponding process conditions. The application of immobilized, instead of free enzyme, avoided the presence of protein in the dyebath, thereby preventing any interaction between denaturated protein and dye (Tzanov *et al.* 2001b). The dyeing results showed that the washing after the second box was not necessary, considering 1 CIELab unit as commercially acceptable colour difference on dyed fabrics.

Conclusion

In this study catalase immobilized on Al_2O_3 pellets and pastilles was used to degrade H_2O_2 in both column and tank reactors. The column reactor with substrate recirculation was more appropriate for our purpose of application, due to the faster decomposition of the peroxide. For both types of reactor inhibition of the enzyme occurred at substrate concentrations above 1 M. The covalent technique for immobilization resulted in improved pH and temperature performance of the enzyme. The immobilized catalase was more active than the free enzyme, retaining 90% of its initial active at 30 °C, and in the range of pH 6–11. Furthermore, the immobilized catalase can be used at the above temperature and even at higher alkalinity, i.e., pH 12 for about 35 h with 50% loss of activity. The immobilization process rendered the enzyme more resistant to inactivation at storing in alkaline medium pH 11–12, and up to 60 °C. Dyeing in the recycled water provided a greater consistency of colour than occurred using the free enzyme. The recycling of the washing liquor for dyeing might provide considerable savings in water, energy and time.

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